Functional polymorphism in the neuropeptide Y gene promoter (rs16147) is associated with serum leptin levels and waist-hip ratio in women

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Abstract: OBJECTIVE: The neuropeptide-Y (NP-Y) gene is a strong candidate gene in the pathophysiology of obesity-linked behavior, and several single-nucleotide polymorphisms of NP-Y have already been linked to body weight and appetite. However, the results from current studies remain inconclusive. The aim of the present study was to test whether a certain functional genetic variant (SNP rs16147) in the NP-Y promoter gene is associated with serum leptin levels and body fat distribution. METHOD: We genotyped and measured the serum leptin levels of the NP-Y rs16147 polymorphism in 1,097 Caucasian subjects in the context of a population-based, case-control multicenter study. We measured weight, height and waist circumference, from which we then calculated BMI and waist-to-hip ratio (WHR). RESULTS: We found the CT-genotype of the SNP rs16147 to be significantly associated with lower WHRs and higher serum leptin levels in women, compared to homozygote gene carriers. No association between rs16147, WHR and serum leptin levels was found in men. CONCLUSION: Our results provide evidence that the functionally relevant SNP in the NP-Y promoter gene affects body fat distribution and serum leptin levels in women, pointing towards possible behavioral effects of NPY in obesity.

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Functional Polymorphism in the Neuropeptide Y Gene Promoter (rs16147) Is Associated with Serum Leptin Levels and Waist-Hip Ratio in Women

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Key Words
Neuropeptide-Y · Polymorphism · Obesity · Waist-to-hip ratio · Genetics · rs16147 · Leptin

Abstract
Objective: The neuropeptide-Y (NP-Y) gene is a strong candidate gene in the pathophysiology of obesity-linked behavior, and several single-nucleotide polymorphisms of NP-Y have already been linked to body weight and appetite. However, the results from current studies remain inconclusive. The aim of the present study was to test whether a certain functional genetic variant (SNP rs16147) in the NP-Y promoter gene is associated with serum leptin levels and body fat distribution. ***Method:*** We genotyped and measured the serum leptin levels of the NP-Y rs16147 polymorphism in 1,097 Caucasian subjects in the context of a population-based, case-control multicenter study. We measured weight, height and waist circumference, from which we then calculated BMI and waist-to-hip ratio (WHR). ***Results:*** We found the CT-genotype of the SNP rs16147 to be significantly associated with lower WHRs and higher serum leptin levels in women, compared to homozygote gene carriers. No association between rs16147, WHR and serum leptin levels was found in men. ***Conclusion:*** Our results provide evidence that the functionally relevant SNP in the NP-Y promoter gene affects body fat distribution and serum leptin levels in women, pointing towards possible behavioral effects of NPY in obesity.

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Introduction

Obesity is a major health problem with extensive consequences not only for those it affects but also for society as a whole [1]. In particular, central obesity – which is measured using the waist-to-hip ratio (WHR) – is associated with increased risk of diabetes, depression, kidney disease, obesity-related cancers and death from cardiovascular disease [2–5]. However, the mechanisms and pathways underlying central obesity and regulation of body fat distribution are not completely understood. While the genetic risk factors [6] as well as the environmental factors [7–10] underlying obesity are still relatively poorly understood, a better understanding of the complex interactions between physical, endocrinological, genetic and molecular phenotypes is needed [11]. Recent evidence suggests that only around 50% of the variation between individuals in body weight has a genetic basis, but these effects are dominated by polygenetic environmental interactions that reflect many genetic influences affecting spontaneous physical activity, metabolic rate, endocrinological changes and appetite behavior [1]. There are a large number of hormones and neuropeptides involved in regulating the homeostasis of body weight, appetite and food intake. Current research has shown the leptin and neuropeptide-Y (NP-Y) systems to be two of the most interesting and important regulators of appetite and energy homeostasis [12, 13]. Since leptin, an anorexigenic peptide, is secreted by adipocytes in proportion to lipid reserves, serum leptin levels provide feedback about the body's fat stores [14]. Leptin is also involved in the long-term regulation of adiposity, and recent research has suggested that leptin diminishes food intake by signaling satiety in the hypothalamus [14]. In obese individuals, a leptin-resistance mechanism affects leptin's regulatory effect, which explains the positive correlation observed between serum leptin levels and body fat mass [15]. In contrast to leptin, NP-Y is an orexigenic neuropeptide, and it promotes food intake and helps reduce energy expenditure [16]. A recently published study found that changes of plasma levels of anti-NP-Y plasma immunoglobulins are relevant to altered appetite and body weight in patients with depressive disorder [17]. Findings from a recent study suggest that circulating leptin directly engages NP-Y neurons, thus regulating bodyweight homeostasis [18]. Furthermore, melatonin has recently been found to play a role in central appetite regulation by modulating the gene transcription of leptin and NP-Y [19]. Also, there is growing evidence that in addition to their roles regulating food intake and energy expenditure, both peptides (NP-Y and leptin) also closely interact with mesolimbic reward pathways [20, 21]. Since the effects of food intake are self-reinforcing, there has been increased discussion recently as to whether severe obesity and addiction disorders share any common neuronal circuits [5, 22]. Furthermore, gene variations in both systems (NP-Y and leptin) have been found to be associated with central obesity as well as numerous psychiatric conditions [14, 23–25]. Obesity is to an essential degree the consequence and measure of maladaptive eating behavior. Despite neurobiological processes controlling food intake, the interaction of genetic, psychological and endocrinological factors which thus constitute risk factors of weight gain and obesity are complex, but important to investigate [1]. Analysis of the candidate genes that regulate psychological and endocrinological pathways could lead to a better understanding of the pathophysiology of central obesity. To this end, we analyzed whether or not a certain functional genetic variant in the NP-Y-promoter gene (SNP rs16147) is associated with body fat distribution and serum leptin levels in an ethnically homogenous sample of healthy white subjects.

Methods

Data Collection

For the case-control association analysis, we analyzed data from a cohort investigating the phenotypes and genetics of nicotine dependence. Of our subjects, 466 were male, 631 were female (entire sample n = 1,097) and all were genotyped. Data were collected at seven recruitment centers located throughout Germany (Departments of Psychiatry at the Universities of Aachen, Berlin, Bonn, Duesseldorf, Erlangen, Mainz, and Mannheim) between 2007 and 2009. All participants were required to be of German origin and to speak German at a native-speaker level. Only ethnically German subjects were included. Details on the recruitment, testing procedures, inclusion/exclusion criteria and characterization of this multicenter study on nicotine dependence and smoking-related behavior have been published elsewhere [26, 27].

Prospective subjects' conformity to inclusion/exclusion criteria was assessed using a medical examination, a standardized psychiatric interview (SCID-I), questionnaires, drug screenings, alcohol testing and carbon monoxide measurement.

The present study was approved by the ethics committees of all participating centers. Subjects were included in the study only after they had given written, informed consent.

Measures and Testing Procedures

The testing sessions were subjected to a strict timetable and included a standardized 600 kcal meal during a 1-hour lunch break taken at noon. The assessments started at 8:30 a.m. and lasted until 4:30 p.m. for all participants. The absence of psychiatric axis-I comorbidity was verified using the Structured Clinical Interview, which is based on DSM-IV criteria (American Psychiatric Asso-

ciation, 2000). Bodyweight, BMI and waist-hip ratio (WHR) were assessed for all participating subjects according to standard procedures. BMI was calculated as body weight (kg) divided by the square of height (m).

DNA Preparation and Genotyping

Genotyping was performed at the Cologne Center for Genomics at the University of Cologne. DNA from fresh frozen EDTA blood was prepared using a Qiagen FlexiGene DNA Kit according to the manufacturer's instructions and normalized based on RNase P copy number measurement using the TaqMan RNase P assay from Applied Biosystems® (Foster City, Calif., USA). The SNP rs16147, a functional NP-Y promoter variant, was chosen to cover the functional expression of brain NP-Y. Genotyping was performed using SNP stream SNP genotyping assays. Genotyping call rates were 99%. All laboratory procedures were carried out blind to case-control status.

Hormonal Measures

Blood samples were obtained between 2:00 and 4:30 p.m. by venipuncture, and were then anticoagulated with sodium EDTA (1 mg/ml whole blood) and immediately cooled on ice. Plasma was separated by centrifugation with 4,000 *g* and aliquots were frozen immediately and stored at –80 °C until analysis (maximum 6 months). The leptin analyses were performed at the Neurobiological Laboratory of the Department of Psychiatry at the University Hospital of Hamburg.

To measure serum leptin levels, we used a human leptin radioimmunoassay kit (Linco, St. Charlex, Mo., USA). The detection limit was 0.25 ng/ml of plasma; intra- and inter-assay coefficients of variation for 4.9. and 15.7 ng/ml levels were below 8.5%.

Data Analysis

Associations between genotype and gender as well as obesity and gender were evaluated using the χ^2 test. The gender-specific risk of obesity is given in odds ratio and its confidence interval. Due to the fact that all three dependent variables (BMI, WHR and leptin) deviated from a normal distribution (Kolmogorov-Smirnov test for all three variables: $p < 0.001$), we performed nonparametric, Mann-Whitney tests in order to compare the influence of the genotype. The genotypes were divided into two groups: one for homozygotes (CC and TT) and another for heterozygotes (CT). Additionally, we calculated correlative associations using Pearson's correlations (two-tailed). All tested statistics indicating a p value of 0.05 or less were considered significant. We tested for deviation from Hardy-Weinberg equilibrium using Fisher's exact test. The data analysis was performed using SPSS Statistics, version 19.

Results

Group Characteristics

A total of 1,097 subjects were genotyped for SNP rs16147. This total sample was primarily female (631 female, 466 male), aged 34.7 ± 12.8 years. SNP rs16147 did not deviate from Hardy-Weinberg equilibrium (TT = 258, 23.5%; CT = 561, 51.1%, and CC = 278, 25.3%; p = 0.469). Genotype distributions for the study population (women and men) are presented in table 1. The study population's sociodemographic and clinical characteristics have been described in previous papers [27–29].

Table 1. Distribution of genotypes in women and men

rs16147	CC	CT	TT
Women	143 (22.7%)	345 (54.7%)	143 (22.7%)
Men	135 (29.0%)	216 (46.4%)	115 (24.7%)

For the purposes of this study, obesity was defined as a BMI of 30 or greater. Of the participants, 120 had a BMI greater than 30 (10.9% of the sample); broken down by gender, 57 of the women were obese (9%) compared to 63 of the men (13.5%). Obesity was unequally distributed between men and women ($\chi^2 = 5.578$, $p = 0.018$), with men having a higher probability of being obese compared to woman (OR = 1.577, 95% CI 1.08–2.31).

The mean WHR for homozygote allele carriers was 0.834 (± 0.955) compared to 0.819 (± 0.909) for heterozygote allele carriers. The TT and CC genotypes of rs16147 were significantly associated with an increased risk of higher WHRs in comparison to the CT genotype (Mann-Whitney U = 108.760, p = 0.008). This association seems to be gender specific, with homozygote (TT and CC; mean WHR = 0.795 ± 0.083) women having significantly higher WHR than heterozygote (CT; mean WHR = 0.773 ± 0.068) women (Mann-Whitney U = 33.730, p = 0.001). In men this comparison is not significant (Mann-Whitney U = 22.424, p = 0.264). However, comparisons of CC and TT alleles did not show any significant differences (Mann-Whitney U = 5.877, p = 0.728).

The mean BMI in women was 23.481 (± 4.855), and in men it was 25.301 (± 4.210). This difference was found to be significant (Mann-Whitney U = 187.575, $p < 0.001$). However, the mean BMI's of homozygote allele carriers (24.386 ± 4.578) and heterozygote allele carriers (24.170 ± 4.805) did not differ significantly (Mann-Whitney U = 140.927, p = 0.212).

The mean serum leptin levels in homozygote allele carriers was 8.798 ± 8.488 ng/ml, compared to 9.669 ± 9.155 ng/ml in heterozygote allele carriers, which indicates significantly higher serum leptin levels in heterozygote allele carriers (Mann-Whitney U = 152.908, p = 0.025). We found the highest serum leptin levels in heterozygote women (12,516 ± 10,091 ng/ml), which were higher than both homozygote women (11,626 ± 8,695 ng/ml) and homozygote men (homozygote: 5.509 ± 6.928 ng/ml; het-

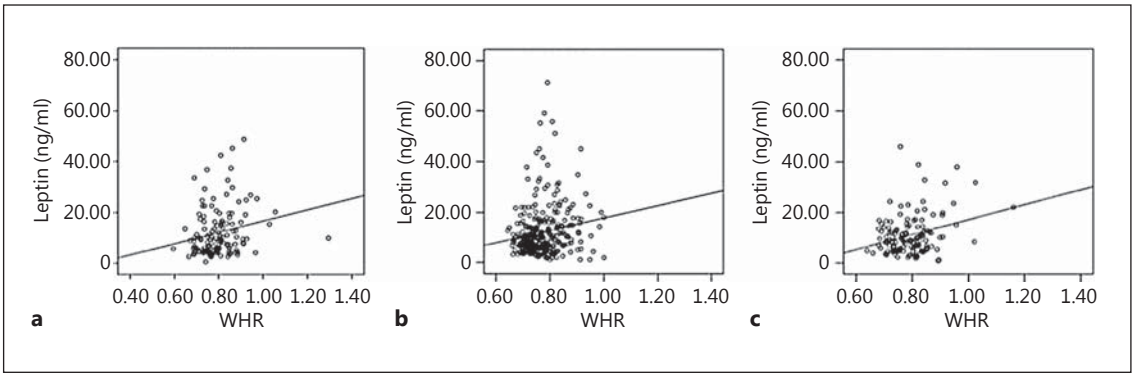

Fig. 1. All genotypes show significant correlation between serum leptin levels and WHR: genotype CC, r = 0.210, p < 0.017 (**a**); genotype CT, r = 0.166, p = 0.004 (**b**); genotype TT, r = 0.290, p = 0.001 (**c**).

erozygote: 5.05 ± 4.50725 ng/ml). Serum leptin levels were 12.115 (± 9.491) ng/ml in women, compared to 5.296 (± 5.921) ng/ml in men (Mann-Whitney U = 55.668, p < 0.001). However, all three genotypes showed significant correlations between serum leptin and WHR (fig. 1).

We conducted further series of correlation analyses, the results of which showed leptin to be significantly associated with BMI (r = 0.474, p < 0.001). In addition, we found serum leptin to be positively correlated with both heart rate (r = 0.061, p = 0.049) and blood pressure (diastolic: r = 0.106, p = 0.001; systolic: r = 0.076, p = 0.015). We also found BMI to be significantly correlated with WHR (r = 0.498, p < 0.001).

We did not observe significant differences between the different alleles of the SNP rs16147 concerning levels of cortisol, ACTH, orexin and cotinin (data not shown).

Discussion

The novel finding of the present study is that the single nucleotide polymorphism rs16147, located in the NP-Y gene promoter, is significantly associated with both WHR and serum leptin concentrations. These associations were sex specific, applying only to women. Our main result is not surprising, since various NP-Y gene variants have recently been found to be associated with obesity [30]. However, the evidence supporting the association between obesity and polymorphisms of the NP-Y gene has been inconsistent. In a large study, the functionally relevant NP-Y SNP rs16147 was not found to be associated with obesity, despite other SNPs covering the NP-Y gene having been found to be associated with obesity (rs17149106, rs16139) [30]. Our study confirmed this result and also found no association between BMI and rs16147. However, we did find rs16147 to be associated with the distribution of the body fat (central obesity). Furthermore, our finding is sex specific. Sexual dimorphism in human body composition is already well known and has been recently described. Compared to women, men are taller, they have lower overall fat mass and their fat distribution, total lean mass and bone mineral mass are also different [31]. Previous studies support our main finding, reporting that the genetic variance for WHR is significantly higher in women than in men [32]. The NP-Y gene thus accounts not only for BMI, as previous studies had suggested. Our results demonstrate that the NP-Y gene also accounts for body fat distribution measured with WHR (an important obesity-related trait), and they also reveal a gene-by-sex interaction.

In a recently published paper by our group using the same database, it could be demonstrated that smokers with a pathological eating behavior show an impaired neuroendocrine regulation of appetite and are prone to experience higher levels of stress and negative affectivity [26]. Altered psychological states (e.g. depression, stress) have been repeatedly associated with impaired neurobiological processes controlling food intake [11, 33–35]. Leptin is an important endocrinological mediator that not only controls for eating behavior, but also acts as an important mediator in stress reactions and depressed mood [36]. One finding of our study is that serum leptin levels are positively correlated with BMI, blood pressure, heart rate and WHR, a finding which has already been reported repeatedly [37]. We found significantly higher serum leptin levels in women with the CT allele compared to female CC- and TT-allele carriers (rs16147). Previous studies had already found leptin levels to be higher in fe-

males than males if leptin levels are expressed as a percentage of body fat [38, 39]. Our result is surprising since most previous studies had found positive associations between leptin and BMI/WHR [40, 41]. However, increased leptin levels have not always been found to be associated with increased bodyweight [42, 43]. As a restriction, it should be noted that in our study we assessed serum leptin levels cross-sectionally, and thus we could not discriminate between acute and chronic hyperleptinemia, although doing so is important for assessing leptin's long-term biological effects.

Taken together, our results suggest that the NP-Y system is involved in body fat distribution in women. We found that individuals carrying the risk genotypes TT and CC of rs16147, which have been found to be associated with altered NP-Y levels in earlier studies [44–46], have both significantly lower serum leptin levels and a higher individual vulnerability to increased WHR. These results were sex specific, implying additional risk factors contributing to the complex phenotype of obesity and WHR. Our study does have some limitations. Firstly, the study findings are not generalizable to all people suffering from obesity as the primary goal of the study was not to study obesity but to investigate tobacco dependence in smokers and nonsmokers. Secondly, we did not investigate whether rs16147 influences NP-Y and leptin expression directly or indirectly, or whether it acts through other pathways – a question that should be addressed in future studies. Furthermore, our study population lacked ethnic diversity, as the analysis was limited to individuals of German ancestry as a means of avoiding the effects of population stratification. For this reason, the study findings may not apply to populations of non-German descent. Finally, there are many nongenetic factors that influence WHR and leptin levels, including circadian rhythms, psychological factors, sex, addiction, as well as other hormones like insulin and cortisol, none of which were taken to account in the present study. The strengths of our analysis include that we found a so far unknown association in the NP-Y gene promoter that may constitute a genetic risk factor for elevated WHR in woman. However, further studies are needed to replicate our preliminary findings.

To our knowledge, this is the first study showing a gene-by-sex interaction with an association between rs16147, serum leptin and WHR. However, the pathogenesis of obesity and obesity-related traits is complex and involves both genetic and environmental factors. Therefore, additional studies looking at different genes, exact (endo-)phenotypes and environmental factors would be useful contributions to this field.

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Disclosure Statement

All authors have no conflict of interest.

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